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(19)(CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Recombinant Protein Which Binds to a Complex Viral Antigen of HIV-1

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Abstract

The invention relates to a recombinant protein which binds to a complex viral antigen of HIV-1. The manufacture, production, purification and application of this protein are described. The protein contains the variable regions of a human anti-HIV-1 antibody connected by a linker. The production is carried out in various prokaryotic or eukaryotic systems. Biochemical chromatographic methods are used for the purification. The described recombinant protein can be used for detecting, quantifying and purifying HIV-1 antigen.

COMPLEX VIRAL ANTIGEN OF RECOMBINANT PROTEIN BINDING HIV-1

Human monoclonal antibodies (mAk) can be produced thereby that B-lymphocytes are obtained from humans which, due for example to illness, show an immune reaction against an antigen and that these B-lymphocytes are immortalized through fusion with suitable cell lines, in particular with myeloma cell lines. Hybrid cell lines, so-called hybridomas, obtained in this way, serve as production vehicle for mAks. They can be utilized in vitro in the form of cell cultures and can be cultivated on the required scale (1).

The substance produced in the process represents as a rule a complete mAk characterized by 2 heavy and 2 light chains, which are linked with each other through disulfide bridges and through non-covalent bonds, and which form the specifically binding antibody (2).

The structure of such an antibody can be divided into a constant region responsible for the so-called effector functions, such as for example complement activation, and into a variable region which brings about the specific binding of the particular antigen.

Biochemical methods can be used to split antibodies enzymatically. For example using papain or pepsin, a portion of the constant region can be split off. The Fab' or $(Fab')_2$ fragments are able in a manner analogous to that of the original antibody, to bind the particular antigen (2). Also described was the proteolytic splitting of the complete constant regions resulting in a so-called Fv fragment. However, the reproducibility of this procedure is not nearly as good as the papin or pepsin splitting of antibodies described above (3,4).

With methods of gene technology it is, however, possible to produce Fv fragments reproducibly. The prerequisites necessary to achieve this as well as the methods utilized will be described in the following.

With the aid of standard methods a cDNA bank of a hybridoma cell line producing mAk is produced. Total RNA is isolated from mAk-producing hybridomas. Apart from ribosomal RNA, this RNA comprises the totality of the transcripts of the cells. Present are incompletely processed, nuclear transcripts as well as mature cytoplamatic transcripts, the so-called messenger RNSa. These are characterized by a poly-adenosine tail at the 3′ end. This poly-a region can be used to isolate the mature mRNAs through affinity chromatography with oligo-dT-cellulose. With the aid of the enzyme "reverse transcriptase" the mRNA can be rewritten into a so-called cDNA. By using suitable vectors, the obtained mixture of cDNAs can be cloned which results in a so-called cDNA bank (5). Immunoglobuline-specific hybridisation probes permit the identification and isolation of clones which comprise the desired sequences. By sequencing the DNA of these clones and by comparing the sequences with known immunoglobuline genes (EMBL Nucleotide Sequence Data Library, Heidelberg, Germany) certainty can be gained about the identity of the clones (5). In this way, clones can be isolated for example which carry the sequences of the light or the heavy chain of an mAk.

Through sequence analysis of the immunoglobuline cDNAs obtained in this way, the individual domains of the heavy or the light chain can be identified by comparison with known immunoglobuline sequences: it is possible to identify the variable and the constant region and, for example, to identify within the variable region the so-

called "hypervariable" or "complementarity determining" regions which, in fact, are responsible for the specific antigen binding (6).

Antibody genes cloned in this way can be brought to expression in different systems. On the one hand, animal cell cultures can be used, such as for example...

myeloma cells if suitable expression vectors are used (7). The use of yeast (8) or of bacterial cells (9) as expression vehicle for complete antibodies is problematic, since such cells are apparently not able to synthesize correctly the - for them - very large molecules such as antibodies represent. Success in this direction took shape when attempts were made to bring to expression subfragments of antibodies in lower eukaryotes or in prokaryotes. In the following four different methods will be described which permit the expression of Fv or Fab fragments in Escherichia coli:

Skerra and Plückthun (1988 (10)) inserted the gene for the variable regions of a murine antiphosphorylcholine - antibody (McPC603) adjoining the lac promoter-operator region followed by one bacterial leader sequence each which served for the transport of the products into the periplasmic space of the bacteria. This is the leader of the outer membrane protein A (opmA) as well as the alkaline phosphatase (phoA). After transfection of this plasmid into Escherichia coli, the expression of functional i.e. antigen-binding protein in the periplasmic space of the bacteria was proven.

Better et al. (1988, (11)) produced the Fab fragment of a chimeric murine-humane antibody which recognizes a ganglioside antigen such as is found frequently on the surface of human carcinoma cells. The herein applied plasmid construction comprises the Salmonella typhimurium araB promoter as well as the pelB leader sequence in each instance before the sequence coding for the particular chain. Antigen-binding Fab fragments were obtained from the culture supernatant of the transformed bacteria.

Interestingly, Skerra as well as Plückthun (1988, (10) and Better et al. (1988, (11)) used so-called dicistronic constructions, i.e. such, in which in a single messenger RNA molecule the information for the two chains to be expressed separately is present. The authors state that thereby the spacial closeness of the forming polypeptide chains is ensured which represents a prerequisit for the correct orientation of the variable region of the heavy (V_H) with that of the light chain (V_L).

Attempts to solve precisely this problem, namely the formation of the Fv peptide heterodimer (in nature not covalently bound) were made by Huston et al. (1988, (12)) and by Bird et al. (1988, (13)) in different ways, namely through covalent linkage of the chain via an amino acid linker sequence such as does not occur in nature. This linker sequence is distinguished thereby

that it comprises a particular number and sequence of amino acids so that it can bridge the space which exists in the natural conformation of an antibody between the regions to be bound, without introducing unnecessary stress into the conformation:

Huston et al. (1988, (12)) linked the variable regions of a murine anti-digoxin antibody via a linker of 15 amino acids having the sequence GGGGSGGGGSGGGGS. The selected order was: V_H - linker - V_L. This so-called single chain Fv fragment was expressed in connection with the MLE leader sequence under the control of the synthetic trp promotor-operator in the form of insoluble inclusion bodies. After their solution in 6 M guanidine HCl and after removal of the leader through acidic hydrolysis between the amino acids Asp and Pro as well as some chromatographic steps, active antigen-binding single chain Fv fragment was obtained.

A basically analogous approach was chosen by Bird et al. (1988, (13)) for the construction of a murine antigen-binding protein which binds fluorescine specifically. This group, however, used a linker of 18 amino acids with the sequence KESGSVSSEQLAQFRSLD. This linker is a part of the sequence of the human "carbonic anhydrase" and was selected from the Brookhaven protein structure data base as a loop structure which spacially fits precisely on the position of the amino acids of the Fv fragment to be bound with each other. The order of the individual regions here was different than with Huston et al. (1988, (12)), namely V_L - liner - V_H.

The above described gene construction for the production of antibody fragments in Escherichia coli refers to murine sequences or in one case to a murine-human chimeres. No corresponding experiments with human sequences have been published.

Fab', $(Fab')_2$ and Fv fragments offer different advantages than do complete antibodies. Due to their small size in comparison to complete antibodies, they can diffuse easier and faster, in vitro as well as in potential in vivo applications. For this reason they are generally easier to work with and in most cases in which the functions of the constant regions (for example effector functions, binding to cell receptors, binding to other molecules) are not required or even present disadvantages, are equivalent to complete antibodies and possibly are even to be preferred. For example, in tumor imaging when using complete antibodies, problems frequently arise

through background signals, which are caused by nonspecific binding of the antibodies to cell receptors transmitted through the constant regions of the antibodies. It is known that when using Fab fragments such problems can be reduced. Accordingly it is to be expected that the use of Fv fragments or of single chain Fv fragments will offer further improvements in this respect (13, 12).

Until now antibodies of murine origin have been worked with which bind to small well described antigens such as fluorescine or digoxin. The entire gene construction builds on the fact that a low molecular substance (MW > 1000) is bound as antigen. The antigenic substances occurring most frequently in nature are peptides, peptidoglycanes, proteins and polysaccharides, and as such are high molecular.

According to the invention the protein of the above stated type comprises the antigen-binding regions of an antibody stemming from the cell line 3D6 (Accession No. 87110301, PHLS, Porton Down, UK (1, 14, 15, 16). Therewith for the first time a protein of human origin is obtained which has the desired binding characteristics and which can also be expressed in unicellular microorganisms such as yeast or bacteria.

Furthermore, according to the present invention the production of a single chain construction, derived from a human antibody is described. This single chain construction binds to an high molecular complex viral antigen, in contrast to small well defined antigens.

It could not have been predicted that the corresponding methods for constructing the single chain fragments would also lead in the case of other than the published antibodies, in particular with human antibodies, to functional, i.e. antigen-binding molecules.

It is further also not obvious, that complex antigens such as for example antigens on the surface of virus, in which according to experience a greater number of amino acids participate in the antigen-antibody formation than with small antigens, tolerate in the same way manipulation in the area of the variable regions of the corresponding binding antibodies.

Starting with the cell line 3D6 which produces a human monoclonal antibody of type IgG1-kappa which reacts specifically with HIV-1 gp41 and shows a weak crossreaction with HIV-1 gp120 (3D6; (1, 14, 15, 16), total RNA was isolated. Therein the method of the guanidine isothiocyanate extraction

and ultracentrifugation via a cushion of 5.7 M CsCl was used (5).

Through adsorption on oligo-dT-cellulose, the poly A + 30 fraction, consequently the mRNA, was isolated from the total RNA, (mRNA Purification Kit, Pharmacia, Sweden).

The mRNA serves as a substrate for the synthesis of cDNA (cDNA Synthesis Kit, Pharmacia, Sweden).

The cloning of the cDNA bank took place in the plasmid vector pUC19. The recombinant plasmid was transformed in Escherichia coli, strain HB101 and cultivated in LB medium (5).

Positive clones were identified through hybridization with specific oligonucleotide probes. The sequences for the probes were taken from the EMBL DNA sequence data base from constant regions of human IgG1 heavy or kappa light chains.

The clones identified through positive hybridization signals were further characterized by restriction analysis and those clones which carry the plasmids with the longest inserts were identified.

Through sequence analysis of these clones one clone each with the complete coding region for the heavy or for the light chain of the antibody was identified. These clones carry the identification pUC3D6HC (SEQ ID No: 1) or pUC3D6LC (SEQ ID NO:2).

Example 1

In the sequence of the inserts of the clones pUC3D6HC (SEQ ID NO:1) or pUC3D6LC (SEQ ID NO: 2) the transition sites between the region of the leader peptide and the variable region as well as between the variable region and the constant region was identified. Through oligonucleotide-directed mutagenesis (in vitro mutagenesis system, Amersham, UK) the following mutations were carried out at these transition sites (see also A-D):

1) Recognition sequences for particular restriction enzymes were mutated in. With the aid of these restriction sites the variable regions of the heavy or the light chain of the antibody 3D6 were cut out of the particular plasmids.

2) The start and stop codons required for subsequent expression were mutated in.

In order to be able to link the variable regions of the antibody 3D6 with a linker, two synthetic oligonucleotides were produced which form the two DNA strands of the linker. The two oligonucleotides were selected so that if they hybridize with one another, a double strand is formed at whose ends overhanging single strand DNA regions are present which correspond precisely to those overhanging ends which are generated when cutting with the corresponding restriction enzymes at the above stated mutated-in restriction sites. This permits the ligation of the variable regions isolated with the aid of this restriction enzyme with the synthetic oligonucleotides of the linker.

```
     -->           LEADER  | VARIABLE REGION    -->
     -->K   G   V   Q   C | E   V   Q   L   V  -->
 141 AAA GGT GTC CAG TGT GAA GTG CAG CTG GTG 170   wild type
     AAA GAA TTC CCC ATG GAA GTG CAG CTG GTG       mutated
          ** *    ** ***
         EcoRI    NcoI
                    Start
```

A: Mutation at the transition between the leader region and the variable region of the heavy chain of the antibody 3D6 (SEQ ID NO:1). Mutated bases are denoted by "*". The coded amino acids in the wild tpe DNA are given, furthermore the mutation-generated restriction sites EcoRI and NcoI as well as the start codon ATG.

```
     --> variable region   | constant region -->
     --> V   T   V   S   S | A   S   T   K   G -->
 519 GTC ACC GTC TCT TCA GCC TCC ACC AAG GGC 548 wild type
     GTC ACC GTC TCT TCA GGA TCC ACC AAG GGC     mutated
                          **
                        BamHI
```

B: Mutation at the transition between the variable region and the constant region of the heavy chain of the antibody 3D6 (SEQ ID NO: 1). Mutated bases are denoted by "*". The coded amino acids in the wild type DNA are given, furthermore the restriction site BamHI generated by the

mutation.

```
     --> leader region     |  variable region    -->
    --> P   G   A   K   C | D   I   Q   M   T  -->
79  CCA GGT GCC AAA TGT GAC ATC CAG ATG ACC 108
    CCA GGT GCC AAA GTC GAC ATC CAG ATG ACC
                        ***
                        SalI
```

C: Mutation at the transition between the leader region and the variable region of the light chain of the antibody 3D6 (SEQ ID NO: 2). Mutated bases are denoted by "*". The coded amino acids in the wild type DNA are given, furthermore the restriction site SalI generated by the mutation.

```
     --> variable region   |  constant region   -->
     --> V   D   I   K   R | T   V   A   A   P  -->
397  GTG GAT ATC AAA CGA ACT GTG GCT GCA CCA 426
     GTG GAT ATC AAA CGA TAA GCT TCT GCA CCA
                         ***  ** *
                          HindIII
                         Stop
```

D: Mutation at the transition between the variable region and the constant region of the light chain of the antibody 3D6 (SEQ ID NO: 2). Mutated bases are denoted by "*". The coded amino acids in the wild type DNA are given, furthermore the restriction site HindIII generated by the mutation as well as the stop codon TAA.

Through ligation of the 3 appropriately pretreated fragments (V_H, linker, V_L) with one another a gene was obtained which at the transition sites between the variable regions and the linker still carried the mutated-in restriction site, which contains nucleotides not corresponding to the nucleotides naturally occurring at these sites. Thereby also a changed amino

acid sequence (see E and F) resulted.

In order to restore the original amino acid sequence at the stated transition sites, the desired DNA sequence was produced (see E and F) through a repeated mutation process.

```
    -->                VH | linker    -->
                          |  * **
380 GTC ACC GTC TCT TCA GGA TCC GGT GGC TCG GGC 412
    GTC ACC GTC TCT TCA GGT GGC GGT GGC TCG GGC
-->  V   T   V   S   S   G   G   G   G   S   G -->
```

E: DNA sequence of the linkage site VH linker before and after the back mutation for the restoration of the natural amino acid sequence in the area of the VH region (SEQ ID NO: 3). Mutated bases are denoted by "*". The final amino acid sequence is given.

```
    -->             linker |  VL           -->
                        ***|
422 TCG GGT GGC GGC GGA GTC GAC ATC CAG ATG   451
    TCG GGT GGC GGC GGA TCT GAC ATG CAG ATG
-->  S   G   G   G   G   S   D   I   Q   M  -->
```

F: DNA Sequence of the linkage site linker - VL before and after the mutation back for the restoration of the natural amino acid sequence in the area of the VL region (SEQ 12D NO: 3). Mutated bases are denoted by "*". The final amino acid sequence is given.

Consequently, with the aid of this methods a gene was constructed having the structure V_H - linker - V_L. This construct is described as sc3D6 (single chain 3D6) and was inserted in the cloning vector pUC19 (SEQ ID NO: 3). The resulting vector carries the identification **pUCsc3D6**.

The sc3D6 gene was cut out of the plasmid pUCsc3D6 through restriction

enzymes and inserted into the bacterial expression vector pKK223-3 (Pharmacia) which comprises the tac promoter inducible with isopropyl beta-thiogalactoside (IPTG). The resulting vector carries the identification pKKsc3D6 and was transformed into the E. coli strain JM105.

Cultivation of the bacteria

The transformed bacteria were cultivated in a laboratory fermenter up to an OD_{600} of 2.0 in LB culture medium (5). Subsequently, the induction of the expression took place through the addition of isopropylthiogalactoside (IPTG). The bacteria were continued to be cultivated for 3 hours in the presence of IPTG, subsequently harvested through centrifugation and stored at -80°C. Subsequently the protein was extracted and purified.

Extraction and purification

For each experimental preparation 10 g biomass (wet weight) were used. The cells were disrupted by means of lysozyme in combination with osmotic shock and subsequently frozen at -20°C. The frozen E. coli paste is broken into small pieces and a 10% suspension is prepared with STE buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 8.0). To this suspension a lysis cocktail comprising nucleases, lysozymes and inhibitors is added (see Table 1).

This E. coli suspension is incubated for 15 minutes at 42°C. Through the addition of triton-X-100 (final concentration 0.5%) and a renewed incubation of 5 minutes at 42°C the cells are lysed.

Harvesting the inclusion bodies

The sediment is resuspended in STE buffer and stirred for 8 hours at 4°C. The inclusion bodies are concentrated through centrifugation. To this end a glycerine cushion (50% glycerine in phosphate buffered saline (PBS) is placed into centrifuge tubes, a layer of an equal volume of suspension is placed over it and the tubes are centrifuged (30 minutes, 6000 rpm, 4°C, JA-20 rotor, J2-21 centrifuge, manufacturer: Beckman).

Dissolving the inclusion bodies

The concentrated inclusion bodies are dissolved in 6 M GuHCl (guanidine hydrochloride) in PBS, pH 8.3 under stirring at 4°C (12 hrs). Subsequently the protein content is determined photometrically.

Refolding

The protein dissolved in GuHCl is refolded in the presence of foreign proteins. First a protein determination takes place. The dissolved inclusion bodies are diluted with refolding buffer (GuHCl 1 M, glutathion reduces 30 mM, glutathion oxidizes 3 mM, EDTA 100 µM, in PBS, pH 8.3) in such a way that the final concentration is 80 mg protein/l.

Diluting the dissolved inclusion bodies takes place on a laboratory scale using a burette by slowly dripping the protein solution into the refolding buffer. It is best to work at 37°C.

The refolding was pursued by means of reversed phase HPLC. To this end samples were taken, the pH value set to 5.5, in order to prevent further refolding, the samples were centrifuged (Millipore table centrifuge, 4700 rpm, room temperature), filtered steril (pore size 0.22 µm, low protein binding), and, if necessary, concentrated (Millipore table centrifuge, 4700 rpm, 20°C) and in each instance 250 µl analysed by means of reversed phase chromatography HPLC (nucleosil 300, 5 µm, 4x125 mm, manufacturer Vogel, Germany. A linear gradient 0.1% TFA / acetonitril 10 - 60 % was applied on the column within 40 minutes).

The folded sc3D6 was ultrafiltered. A 10000 Dalton cutoff polysulphone membrane was used. The ultrafiltered protein solution is placed on an anion exchanger and subsequently eluted from the column with 100 mM NaCl.

The sc3D6 is desalinated with Sephadex G-25 (manufacturer: Pharmacia, Sweden) gel filtration and conjugated with alkaline phosphatase according to the method by Nakane et al. (17).

The purified sc3D6 protein was examined through SDS PAGE (Illustration 7). For demonstration of the functionality of the sc3D6 a Western Blot Test with HIV-1 test strips (BioRad, USA) was carried out. As positive control an analogous test was carried out with the natural antibodies isolated from

animal cells. As negative control served a preparation of total protein from E. coli. The result of this test was positive and is shown in Illustration 8.

Purification of sc3D6 protein by means of affinity chromatography

With the appropriately purified sc3D6 protein a rabbit serum was produced under standard conditions with complete Freund's Adjuvans. With the aid of CM-Sepharose Fast Flow Chromatography (Manufacturer Pharmacia, Sweden) the IgG fraction was obtained from the rabbit serum. The specificity of the antibodies was determined by means of ELISA. With the aid of a peptide synthesizer the 15 amino acid long linker peptide (Sequence: GGGGSGGGGSGGGGS) was produced and subsequently conjugated by means of carbodiimide condensation with bovine serum albumin (BSA) in a molar ratio of 6:1. With this conjugate microtiter plates were coated. The serum sample was incubated in the coated microtiter plates and the bound antibody was demonstrated with a peroxidase-marked goat anti rabbit IgG. The anti sc3D6 produced and checked was bound to a BrCN-activated Sepharose 4B (manufacturer: Pharmacia, Sweden). The nonbound material was washed out. A prepurified extract of sc3D6 protein, which was refolded and desalinated with Sephadex G-25 (manufacturer: Pharmacia, Sweden) as described above was placed onto the anti-sc3D6 column. The nonbound material was washed out and the specifically bound sc3D6 protein was eluted with a 0.1 M glycine HCl buffer, pH 2.5. The eluate was subsequently neutralized with 1 M Tris buffer and sc3D6 protein as described characterized by means of SDS electrophoresis and examined for functionality by means of Western Blot.

Another method for the immune affinity chromatographic purification of sc3D6 protein is as follows:

With the above described BSA-coupled linker peptide a rabbit serum was produced with the aid of complete Freund's adjuvans. The IgG fraction was obtained through CM-Sepharose Fast Flow Chromatography (manufacturer: Pharmacia, Sweden) and further purified via a BSA-Sepharose 4B column (manufacturer: Pharmacia, Sweden) in order to remove the anti-BSA antibodies. The so obtained anti-linker IgG was coupled to an BrCN-activated Sepharose 4B (manufacturer: Pharmacia, Sweden). A prepurified extract of sc3D6 protein

which was refolded and desalinated with Sephadex G-25 (manufacturer: Pharmacia, Sweden) as described above was placed onto the anti-linker column. The nonbound material was washed out and the specifically bound sc3D6 protein was eluted with an 0.1 M glycine HCl buffer, pH 3.0. The eluate was subsequently neutralized with 1 M Tris buffer and the sc3D6 protein was, as described, characterized by means of SDS electrophoresis and examined for functionality by means of Western Blot.

Immunoaffinity chromatographic purification of HIV-1 gp160

For the production of an sc3D6 immunoaffinity column the purified sc3D6 protein was bound to a 1 ml NHS column (manufacturer: Pharmacia, Sweden) (acc. to protocol of Pharmacia).

The prepurification of the gp160 (of the coat protein of HIV-1) which is specifically bound by the antibody 3D6 as well as by the sc3D6 protein) was carried out according to Barrett et al. (18).

The prepurified material comprising the recombinant gp160 was concentrated by ultrafiltration and conditioned for the sc3D6 immunoaffinity chromatography. This conditioned material was placed onto the sc3D6 immunoaffinity column. As equilibration buffer a 100 mM Tris buffer, pH 7.4 with 0.1% Tween 20 was used. The recombinant antigen was eluted with 3 M rhodanide. The yields of the individual stages are summarized in Table 2.

Example 2

A further cloning of the sc3D6, in which the sc3D6 gene was fused with the gene for alkaline phosphatase (EcphoA) isolated from Escherichia coli, was carried out as follows:

The sc3D6 gene was cut from the plasmid pUCsc3D6 through restriction enzymes and inserted in the vector pEcphoAMut3 (19). The resulting vector carries the identification **pAPsc3D6**. The vector pEcphoAMut3 contains the gene for alkaline phosphatase (20) isolated from Escherichia coli in which a restriction site was mutated in through oligonucleotide-directed mutagenesis at the 3' end of the coding region, which permits the fusion of the EcphoA gene with other genes. In this way through expression of a fusion gene

fusion proteins are produced i.e. proteins in which the particular coding regions are linked through peptide bonds via amino acids.

The EcphoA - sc3D6 fusion gene was cut from pAPsc3D6 with restriction enzymes and inserted into the bacterial expression vector pKK223-3 (manufacturer: Pharmacia, Sweden). The resulting plasmid is identified as **pKKAPsc3D6.**

The plasmid pKKAPsc3D6 was transformed into the Escherichia coli strain JM 105 and the transformed bacteria cultivated in LB nutrient medium (5). After induction with IPTG active EcPhoA - sc3D6 fusion protein from the periplasmic space of the bacteria was purified as follows:

The bacteria were harvested through centrifugation and washed in 10 mM Tris buffer, pH 7.5 to which had been added 30 mM NaCl. The washed bacteria were resuspended in 33 mM Tris buffer, pH 7.5 and mixed with an equal volume of 40% saccharose solution in (33 mM Tris buffer) and EDTA was added to a final concentration of 0.1 mM. After incubation for 10 minutes at room temperature the bacteria were centrifuged off and placed into 0.5 mM $MgCl_2$ solution. After an incubation time of 10 minutes at 0°C a protease inhibition cocktail comprising PMSF and EGTA was added and the bacteria centrifuged off. The supernatant is brought with 1 M Tris solution, pH 7.5 to a final concentration of 25 mM Tris. Through this procedure the periplasmic space of the E. coli cells is exposed.

Through centrifugation at 12000g the protein solution is clarified and subsequently concentrated through ultrafiltration.

The EcPhoA - sc3D6 protein is further purified with hydrophobic interaction chromatography. A Phenylsepharose Fast Flow (Pharmacia, Sweden) was equilibrated with 60% saturated ammonium sulfate solution in 25 mM Tris buffer, pH 7.5. The protein solution was placed onto the column alternating with the equilibration buffer. The sc3D6 is eluted with a linear gradient of 60% ammonium sulfate to 0% ammonium sulfate. The fractions which contain the EcPhoA - sc3D6 protein are desalinated through gel filtration.

After proving the functionality of the EcPhoA - sc3D6 protein, a Western Blot with HIV-1 test strips (BioRad, USA) was carried out. As a control, an analogous test was carried out with the natural antibody isolated from animal cells. As negative control served a preparation of total protein from E. coli. The result of this test was positive and is shown in Illustration 8.

Direct proof of HIV-1 antigen by means of ELISA

A gp120-specific monoclonal antibody (Clone 25 C2, Accession No. 89120601, PHLS, Porton Down, UK) was coated onto microtiter plates (grade I, Nunc, Denmark). HIV-1 containing culture supernatant (16) was placed onto the coated microtiter plates. Recombinant gp160 (18) was used as standard.

After rinsing out the nonbound material, the EcPhoA-sc3D6 protein was applied and incubated. The nonbound material was again rinsed and the bound EcPhoA-sc3D6 protein was demonstrated photometrically at 602 nm with p-nitrophenylphosphate. In Illustration 9 the standard curve and different samples of HIV-1 positive cultur supernatant are depicted.

Competitive Anti-HIV-1 ELISA

Microtiter plates (grade I, Nunc, Denmark) were coated with a solution of 10 mg/ml recombinant gp160 (18). Subsequently the plates were washed with PBS + 0.1% Tween 20 + 1% BSA.

A solution of 5 µg/ml EcPhoA - sc3D6 fusion protein was mixed at a ratio of 1:1 with HIV-1 positive or HIV-1 negative serum and placed onto the coated plates. As control EcPhoA - sc3D6 fusion protein mixed with dilution buffer was applied and incubated at 37°C for 60 minutes. The nonbound material was subsequently rinsed.

Through the addition of p-nitrophenylphosphate the fraction of bound EcPhoaA - sc3D6 protein was demonstrated. The resulting color was quantified photometrically at 602 nm. The inhibition of the sera was determined as percent of the extinction of EcPhoA - sc3D6 protein without serum. As depicted in Illustration 10, all HIV-1 positive sera inhibit the binding of EcPhoA - sc3D6 fusion protein to gp160. All HIV-1 negative sera showed less inhibition than the HIV-1 positive sera.

Example 3

Another expression type for the sc3D6 protein, in which mouse myeloma cells were used as host cells, was carried out as follows:

The 3' portion of the sc3D6 gene was isolated from the plasmid pUCsc3D6 (SEQ ID NO: 3) through partial EcoRV digestion as well as through complete HindIII digestion (length of the fragment: 401 bp). From the plasmid pUC3D6HC (SEQ IF NO: 1) the 3' portion of the gene for the heavy chain was removed by cutting with EcoRV and HindIII. Into the remaining vector the 401 bp fragment of the sc3D6 gene isolated through agarose gel electrophoresis and purified was inserted. The gene recombined in this way consequently comprises the sequence for the leader peptide of the heavy chain of the antibody 3D6 followed by the sequence of the sc3D6 gene. The plasmid carries the identification pLsc3D6. This construction permits the transport of the sc3D6 protein in animal cells. The coding gene was isolated from pLsc3D6 with the enzymes NcoI and HindIII, the overhanging ends were filled with Klenow polymerase and cloned into the SmaI site of the expression vector pRcRSV (Invitrogen, USA) suitable for animal cells. The SmaI site of this expression vector lies between the long terminal repeat of RSV, consequently a strong viral promoter, and transcription termination sequences which stem originally from bovine growth hormon. Through insertion into this restriction site it is possible to bring any structure gene into a molecular environment which permits the expression of the genes in animal cells. Moreover, the vector pRcRSV additionally has a selection marker "neomycin resistance" which permits the selection of successfully transformed animal cells in the culture.

The plasmid constructed in this way carries the indentification pRcRSVLsc3D6. It was transfected into mouse myeloma cells of line P3-X63-Ag8.653 (21). A total of 5 clones were selected after selection of transformed cells with the antibiotic neomycin in 2 cloning and screening rounds which expressed the sc3D6 gene. The expression level of the individual clones were tested by means of antigen-specific ELISA. The yields of the individual purification stages werde determined by means of antigen-specific ELISA and are between 0.5 and 1 µg/ml.

The culture supernatant containing the sc3D6 protein of the transfected mouse myeloma cells was clarified by centrifugation at 5000 g in a bucket centrifuge. The clarified supernatant was concentrated by the 10-fold through ultrafiltration (Minitan, PTGC, cut-off 10000 Dalton, manufacturer Millipore) and diafiltered with a 50 mM Tris buffer, pH 7.2 with the five-

fold volume.

The diafiltered protein solution was further purified with Q-Seraphose Fast Flow (manufacturer: Pharmacia, Sweden) (equilibration buffer 50 mM Tris buffer, pH 7.2). The elution of the sc3D6 protein took place with 150 mM NaCl. The purified protein was tested by means of antigen-specific ELISA. The yields of the individual purification stages are shown in Table 3.

Example 4

The plasmid pRcRSVLsc3D6 was transfected into Chinese hamster ovary (CHO) cells. In analogous manner to that described in Example 3, transformed cells were selected and screened and the sc3D6 protein from the cell culture supernatant was purified. Testing of the expression levels by means of antigen-specific ELISA brought values between 1 and 5 µg/ml antibodies.

Example 5

The sc3D6 gene was cut from the plasmid pUCsc3D6 through restriction enzymes and inserted into the yeast expression vector pG1 (Clontech Laboratories Inc., Palo Alto, USA). In this construction the sc3D6 gene was placed under the regulation of the GAL1 promoter inducible through galactose. The construct was transfected into the saccharomyces cerevisiae strain SHY2 (trp1) and selected in medium without tryptophane for complementation of the tryptophane auxotrophy. Positive transformants were isolated and utilized for the production of sc3D6 protein. The conditions for the cultivation of the production strain as well as for the isolation, preparation and purification of the product were carried out according to standard protocols (22).

Example 6

The sc3D6 gene was cut from the plasmid pUCsc3D6 through restriction enzymes and inserted into the vector pAc373 (23). This recombinant plasmid was transfected together with DNA of the baculovirus autographa californica nuclear polyhedrosis virus (AcMNPV) into the cell line Sf9 stemming from

spodoptera frugiperda. The cultivation of the Sf9 cells took place according to the standard method described in the Catalogue of the American Type Culture Collection. 3 to 5 days after the transfection plagues of recombinant virus were microscopically identified and isolated. In order to be sure that the isolated recombinant virus were not contaminated with wild type virus, three further plaque purification processes followed. Infection of Sf9 cells with recombinant virus after 3 to 5 days lead to lysis of the infected cells and, concomitantly with it, to the production of sc3D6 protein in the supernatant of the cell lysate. The sc3D6 protein was, as described analogously in Example 3, purified and analysed. In this way the functionality of this recombinant protein could be demonstrated.

Example 7

The sequence (24) coding for the protein avidin was produced as synthetic gene through synthetic oligonucleotides and specifically so that additionally at the 5' end of the gene the sequence of the leader peptide for E. coli alkaline phosphatase (20) and at the 3' end a polylinker region for inserting other genes is present. Genes inserted in this polylinker region are expressed under suitable conditions as fusion proteins with avidin as fusion partner. With the aid of the leader located at the 5' end, these fusion proteins were transported in active form into the periplasmic space of Escherichia coli. This construct was inserted into a suitable restriction site of the bacterial expression vector pET-3a (25) which contains for the expression of cloned genes the bacteriophage T7-ϕ10 promoter as well as the ϕterminator. The resulting vector carries the identification pET-3a-Av.

The bacteriophage T7-ϕ10 promoter has the characteristic of not being transcribed in E. coli cells in the absence of the bacteriophage T7 RNA polymerase. If, however, for example a densely grown E. coli culture is infected with a phage vector which carries the genetic information for the T7 polymerase, the thereby produced T7 polymerase leads to the expression of genes, which for example in vectors such as those described above are present in cloned form. This characteristic is very important for the expression of avidin and avidin fusion proteins in E. coli since the avidin is toxic for growing E. coli cultures.

The sc3D6 gene was cut from the vector pUCsc3D6 through restriction enzymes and inserted into the polylinker region of the vector pET-3a-Av. The resulting vector carries the identification pET-3a-Av-sc3D6. Suitable E. coli host cells (for example HMS174) were transformed with this vector and cultivated. As soon as the culture had reached an OD_{600} of 0.6, infection was carried out with the bacteriophage CE6 (lambda cIts857Sam7) (25) which carries the bacteriophage T7 gene1. The thereby formed T7 polymerase led to the expression of the avidin-sc3D6 fusion protein in the periplasmic space of the E. coli. As soon as the expression had reached its maximum (depending on the culture conditions between 3 and 12 hours after infection with the phage), the recombinant protein was, analogously to the manner described in Example 2, freed and concentrated through ultrafiltration.

The concentrated protein solution was further purified over Sephacryl S 200 (Pharmacia) and concentrated for a second time by means of ultrafiltration. This solution is placed onto a biotin column. The corresponding fusion protein avidin-sc3D6 remains specifically bound. The impurities are rinsed out. The affinity column prepared in this way was used for the purification of recombinant gp160 analogously to Example 1, i.e. the protein solution prepurified according to Barrett et al. (18) was placed onto the affinity column and after rinsing out the nonbound material the recombinant gp160 was eluted with 3 M rhodanide. The yields achieved thereby are analogous to the results shown in Table 2.

SEQ ID NO: 1
Type of Sequence: nucleotides with corresponding protein
Length of sequence: 1548 base pairs
Strand form: single strand
Type of molecule: plasmid DNA with insert of human cDNA

Origin:
Organism: human
Immediate experimental origin:
Name of cell line: 3D6

Characteristics:

from	1 to	36	bp	plasmid pUC19 polylinker
	37	1527		insert heave chain of antibody 3D6
	37	98		5' nontranslated region
	99	1526		coding region
	99	155		signal peptide
	156	1526		mature peptide
	156	533		variable region
	156	245		framework 1
	246	260		complementarity determining region 1
	261	302		framework 2
	303	353		complementarity determining region 2
	354	449		framework 3
	450	500		complementarity determining region 3
	501	533		framework 4
	534	1526		constant region
	1527	1547		plasmid pUC 19 polylinker

Characteristics: cDNA clone of the heavy chain of the antibody 3D6 inserted into the plasmid pUC19.

```
GTGAATTCGA GCTCGGTACCC GGGGATCCTC TAGAGTCCCA GCCCTGAGAT TCCCAGGTGT    60
TTCCATTCAG TGATCAGCACT GAACACAGAG GACTCACC                            98

ATG GAG TTG GGA CTG AGC TGG ATT TTC CTT TTG GCT ATT TTA AAA          143
MET Glu Leu Gly Leu Ser Trp Ile Phe Leu Leu Ala Ile Leu Lys
                -15                 -10                  -5

GGT GTC CAG TGT GAA GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG          188
Gly Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
                  1                   5                  10
```

GTA CAG CCT GGC AGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA 233
Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
15 20 25

TTC ACC TTT AAT GAT TAT GCC ATG CAC TGG GTC CGG CAA GCT CCA 278
Phe Thr Phe Asn Asp Tyr Ala MET His Trp Val Arg Gln Ala Pro
30 35 40

GGG AAG GGC CTG GAG TGG GTC TCA GGT ATA AGT TGG GAT AGT AGT 323
Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Ser Trp Asp Ser Ser
45 50 55

AGT ATA GGC TAT GCG GAC TCT GTG AAG GGC CGA TTC ACC ATC TCC 368
Ser Ile Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
60 65 70

AGA GAC AAC GCC AAG AAC TCC CTG TAT CTG CAA ATG AAC AGT CTG 413
Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln MET Asn Ser Leu
75 80 85

AGA GCT GAG GAC ATG GCC TTA TAT TAC TGT GTA AAA GGC AGA GAT 458
Arg Ala Glu Asp MET Ala Leu Tyr Tyr Cys Val Lys Gly Arg Asp
90 95 100

TAC TAT GAT AGT GGT GGT TAT TTC ACG GTT GCT TTT GAT ATC TGG 503
Tyr Tyr Asp Ser Gly Gly Tyr Phe Thr Val Ala Phe Asp Ile Trp
105 110 115 120

GGC CAA GGG ACA ATG GTC ACC GTC TCT TCA GCC TCC ACC AAG GGC 548
Gly Gln Gly Thr MET Val Thr Val Ser Ser Ala Ser Thr Lys Gly
125 130 135

CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG 593
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
140 145 150

GGC ACA GCA GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA 638
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
155 160 165

CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG 683
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
170 175 180

CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC 728
His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
185 190 195

AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC 773
Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
200 205 210

TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC 818
Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
215 220 225

AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA 863
Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
230 235 240

CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC 908
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
245 250 255

TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT 953
Phe Pro Pro Lys Pro Lys Asp Thr Leu MET Ile Ser Arg Thr Pro
260 265 270

GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG 998
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
275 280 285

GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC 1043
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
290 295 300

AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC TCC ACG TAC CGT GTG 1088
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305 310 315

GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG 1133
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
320 325 330

GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC 1178
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
335 340 345

GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG 1223
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
350 355 360

GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG 1268
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
365 370 375

GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC 1313
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
380 385 390

GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG 1358
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
395 400 405

ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC 1403
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
410 415 420

AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC 1448
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
425 430 435

TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA 1493
Phe Ser Cys Ser Val MET His Glu Ala Leu His Asn His Tyr Thr
440 445 450

CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA 1526
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Stop
455 460

GACCTGCAGG CATGCAAGCT T 1547

SEQ ID NO: 2
Type of Sequence: nucleotides with corresponding protein
Length of sequence: 945 base pairs
Strand form: single strand
Topology: circular
Type of molecule: plasmid DNA with insert of human cDNA

Origin:
Organism: human
Immediate experimental origin:
Name of cell line: 3D6

Characteristics:

from	1 to	21	bp	plasmid pUC19 polylinker
	22	732		insert light of antibody 3D6
	22	27		5' nontranslated region
	28	732		coding region
	28	93		signal peptide
	94	732		mature peptide
	94	408		variable region
	94	162		framework 1
	163	195		complementarity determining region 1
	196	240		framework 2
	241	261		complementarity determining region 2
	262	357		framework 3
	358	378		complementarity determining region 3
	379	408		framework 4
	409	732		constant region
	733	905		3' nontranslated region
	906	945		plasmid pUC 19 polylinker

Characteristics: cDNA clone of the light chain of the antibody 3D6 inserted into the plasmid pUC19.

```
GTGAATTCGA GCTCGGTACC CCACAGC                                27
ATG GAC ATG AGG GTC CCC GCT CAG CTC CTG GGG CTC CTG CTG CTC  72
MET Asp MET Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu
            -18                 -13                 -8

TGG CTC CCA GGT GCC AAA TGT GAC ATC CAG ATG ACC CAG TCT CCT  117
Trp Leu Pro Gly Ala Lys Cys Asp Ile Gln MET Thr Gln Ser Pro
                -3                  3                   8
```

TCC ACC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC 162
Ser Thr Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
13 18 23

CGG GCC AGT CAG AGT ATT AGT AGG TGG TTG GCC TGG TAT CAG CAG 207
Arg Ala Ser Gln Ser Ile Ser Arg Trp Leu Ala Trp Tyr Gln Gln
28 33 38

AAA CCA GGG AAA GTC CCT AAG CTC CTG ATC TAT AAG GCA TCT AGT 252
Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr Lys Ala Ser Ser
43 48 53

TTA GAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG 297
Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly
58 63 68

ACA GAA TTC ACT CTC ACC ATC AGC AGC CTG CAG CCT GAT GAT TTT 342
Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp Asp Phe
73 78 83

GCA ACT TAT TAC TGC CAA CAG TAT AAT AGT TAT TCT TTC GGC CCT 387
Ala Thr Tyr Tyr Cys Cln Gln Tyr Asn Ser Tyr Ser Phe Gly Pro
88 93 98

GGG ACC AAA GTG GAT ATC AAA CGA ACT GTG GCT GCA CCA TCT GTC 432
Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala Ala Pro Ser Val
103 108 113

TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC 477
Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
118 123 128

TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA 522
Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
133 138 143

GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG 567
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
148 153 158

GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC 612
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
163 168 173

AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA 657
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys
178 183 188

GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC 702
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
193 198 203

ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG 732
Thr Lys Ser Phe Asn Arg Gly Glu Cys Stop
208

CACCTGCTCC TCAGTTCCAG CCTGACCCCC TCCCATCCTT TGGCCTCTGA CCCTTTTTCC 792
ACAGGGGACC TACCCCTATT GCGGTCCTCC AGCTCATCTT TCACCTCACC CCCCTCCTCC 852
TCCTTGGCTT TAATTATGCT AATGTTGGAG GAGAATGAAT AAATAAAGTG AATGGGGATC 912
CTCTAGAGTC GACCTGCAGG CATGCAAGCT TGG 945

SEQ ID NO: 3
Type of Sequence: nucleotides with corresponding protein
Length of sequence: 776 base pairs
Strand form: single strand
Topology: circular
Type of molecule: plasmid DNA with insert of engineered human cDNA

Origin:
Organism: human
Immediate experimental origin:
Name of cell line: 3D6

Characteristics:

from	1 to	13	bp	plasmid pUC19 polylinker
	14	760		insert sc3D66
	14	16		start codon
	14	394		variable region heavy chain
	17	106		framework 1 heavy chain
	107	121		complementarity determining region 1 heavy chain
	122	163		framework 2 heavy chain
	164	214		complementarity determining region 2 heavy chain
	215	310		framework 3 heavy chain
	311	361		complementarity determining region 3 heavy chain
	362	394		framework 4 heavy chain
	395	440		linker
	441	760		variable region light chain
	441	508		framework 1 light chain
	509	542		complementarity determining region 1 light chain
	543	588		framework 2 light chain
	589	607		complementarity determining region 2 light chain
	608	703		framework 3 light chain
	704	724		complementarity determining region 3 light chain
	725	757		framework 4 light chain
	758	760		stop codon
	761	776		plasmid pUC 19 polylinker

Characteristics: Clone of the engineered single-chain Fv fragement of the antibody 3D6 inserted into the plasmid pUC19.

AAAAGAATTC CCC 13

ATG GAA GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTA CAG CCT 58
MET Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro
5 10 15

GGC AGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT 103
Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
20 25 30

```
ATT GAT TAT GCC ATG CAC TGG GTC CGG CAA GCT CCA GGG AAG GGC          148
Asn Asp Tyr Ala MET His Trp Val Arg Gln Ala Pro Gly Lys Gly
                 35                  40                  45

CTG GAG TGG GTC TCA GGT ATA AGT TGG GAT AGT AGT AGT ATA GGC          193
Leu Glu Trp Val Ser Gly Ile Ser Trp Asp Ser Ser Ser Ile Gly
                 50                  55                  60

TAT GCG GAC TCT GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAC          238
Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
                 65                  70                  75

GCC AAG AAC TCC CTG TAT CTG CAA ATG AAC AGT CTG AGA GCT GAG          283
Ala Lys Asn Ser Leu Tyr Leu Gln MET Asn Ser Leu Arg Ala Glu
                 80                  85                  90

GAC ATG GCC TTA TAT TAC TGT GTA AAA GGC AGA GAT TAC TAT GAT          328
Asp MET Ala Leu Tyr Tyr Cys Val Lys Gly Arg Asp Tyr Tyr Asp
                 95                 100                 105

AGT GGT GGT TAT TTC ACG GTT GCT TTT GAT ATC TGG GGC CAA GGG          373
Ser Gly Gly Tyr Phe Thr Val Ala Phe Asp Ile Trp Gly Gln Gly
                110                 115                 120

ACA ATG GTC ACC GTC TCT TCA GGT GGC GGT GGC TCG GGC GGT GGT          418
Thr MET Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
                125                 130                 135

GGG TCG GGT GGC GGC GGA TCT GAC ATC CAG ATG ACC CAG TCT CCT          463
Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln MET Thr Gln Ser Pro
                140                 145                 150
```

TCC ACC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC 508
Ser Thr Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
155 160 165

CGG GCC AGT CAG AGT ATT AGT AGG TGG TTG GCC TGG TAT CAG CAG 553
Arg Ala Ser Gln Ser Ile Ser Arg Trp Leu Ala Trp Tyr Gln Gln
170 175 180

AAA CCA GGG AAA GTC CCT AAG CTC CTG ATC TAT AAG GCA TCT AGT 598
Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr Lys Ala Ser Ser
185 190 200

TTA GAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG 643
Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly
205 210 215

ACA GAA TTC ACT CTC ACC ATC AGC AGC CTG CAG CCT GAT GAT TTT 688
Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp Asp Phe
220 225 230

GCA ACT TAT TAC TGC CAA CAG TAT AAT AGT TAT TCT TTC GGC CCT 733
Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Ser Phe Gly Pro
235 240 245

GGG ACC AAA GTG GAT ATC AAA CGA TAA 760
Gly Thr Lys Val Asp Ile Lys Arg Stop---
250

GCTTCTGCAC CATCTG 776

Tables

Substance	Final Concentration
lysozyme	0.2 mg/ml
RNase	15 U/ml
DNase	15 U/ml
EDTA	100 mM

Table 1: Final concentration of the lysis chemicals in the cell suspension

Stage	Volume (ml)	Protein (mg)	gp160 (mg)	Yield (%)
Extraction	7000	38,200	600	100
Lentil Sepharose	520	1000	372	62
sc3D6 affinity chromatographic	130	148	144	24

Table 2: Yield of the individual stages of the immunoaffinity chromatographic purification of recombinant gp160 with sc3D6 as affinity ligand

Stage	Volume (ml)	Protein (mg)	Titre
Culture supernatant	3500	7600	1:256
Ultrafiltration	350	5300	1:2048
Q-Seraphose	50	72	1:10000

Table 3: Yield of the individual stages of the purification of sc3D6 protein from the culture supernatant of transformed mouse myeloma cells.

WE CLAIM:

1. A recombinant protein binding to a complex viral antigen of HIV-1, characterized in that it comprises the variable regions of an antibody derived from the cell line 3D6.

2. Recombinant protein as stated in Claim 1, characterized in that it comprises the variable region of the heavy chain according to SEQ ID NO: 1.

3. Recombinant protein as stated in Claim 1 or 2, characterized in that it comprises the variable region of the light chain according to SE ID NO: 2.

4. Recombinant protein as stated in one of Claims 1 to 3, characterized in that it is constructed according to SEQ ID NO: 3 wherein the variable region of the heavy chain is connected with the variable region of the light chain through a linker.

5. Process for the production of a recombinant protein as stated in one of Claims 1 to 4, characterized in that a DNA insertion sc3D6 or a sequence hybridized with this insertion or a sequence derived through degeneration from the expressed protein is introduced into a plasmid, transforms a host with this plasmid, and that the construct is expressed.

6. Process for the production of a recombinant protein as stated in Claim 5, characterized in that it is expressed as a fusion protein, in particular together with alkaline phosphatase or together with avidin.

7. Insertion for use in the process as stated in

Claim 5, characterized in that the insertion sc3D6 has the nucleotide sequence given in SEQ ID NO: 3.

8. Process for purifying the recombinant protein as stated in one of Claims 1 to 4, characterized in that specific antibodies against the protein and/or against the linker are emplaced between the two variable portions.

9. Process as stated in Claim 8, characterized in that the antibodies used for the purification are immobilized on a carrier.

10. Process for the isolation and/or purification of HIV-1 antigen, characterized in that the isolation and/or purification takes place through affinity chromatography, wherein, if necessary after suitable prepurification, the sc3D6 protein or the avidin sc3D6 protein is used as ligand for the affinity chromatography.

11. Process for the direct proof of HIV-1 antigen, characterized in that a fusion protein comprising EcphoA sc3D6 protein is utilized as combined detection and signal protein.

12. Process for the proof of HIV-1 positive sera in competitive immune assays, characterized in that a fusion protein is utilized which comprises EcphoA so3D6 protein as combined detection and signal protein.

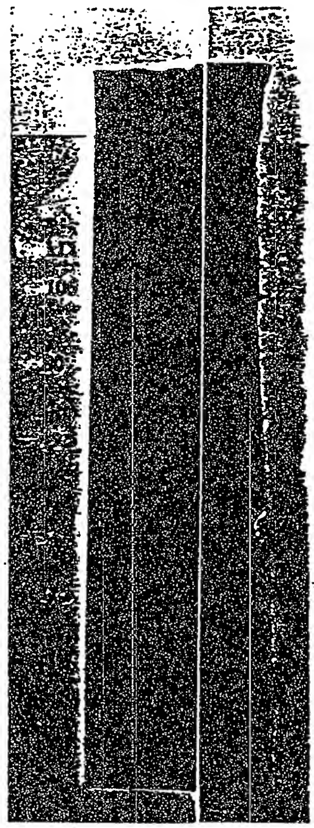

DIAGRAM 1: SDS GEL OF PURIFIED sc3D6. THE MOLECULAR WEIGHTS FOR THE APPLIED STANDARD ARE PROVIDED.

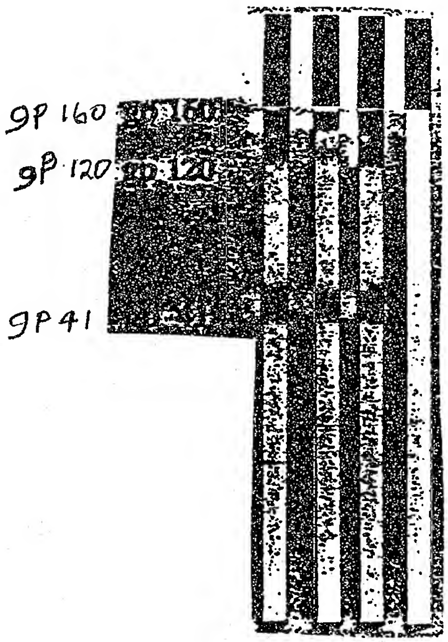

DIAGRAM 2 HIV WESTERN BLOT STRIPS

ANTIGEN BINDING PROTEIN:

1. Sc3D6 PROTEIN
2. APsc3D6 FUSIONSPROTEIN
3. ANTIBODIES 3D6
4. TOTAL PROTEIN FROM E. COLI

EMPERICAL PROOF OF PRESENCE OF HIV-1 ANTIGEN

STANDARD CURVE

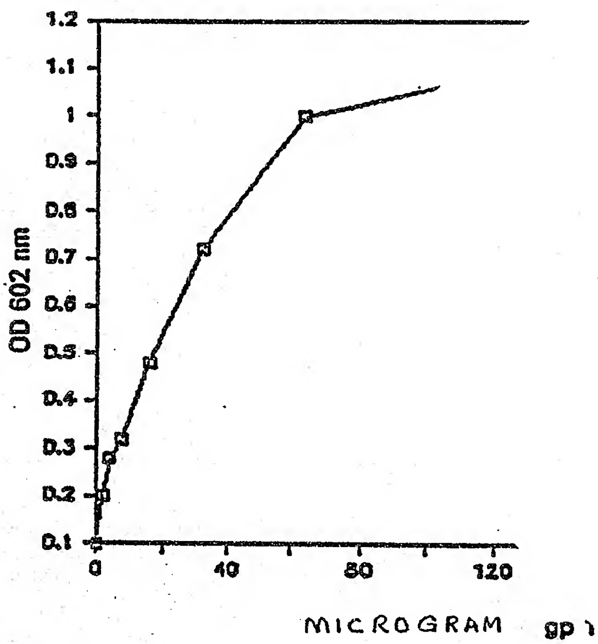

DIAGRAM 3 STANDARD CURVE DEMOSTRATING BY MEANS OF ELISA THE PRESENCE OF HIV-1 ANTIGEN

COMPETIVE ELISA WITH APsc3D6 PROTEIN

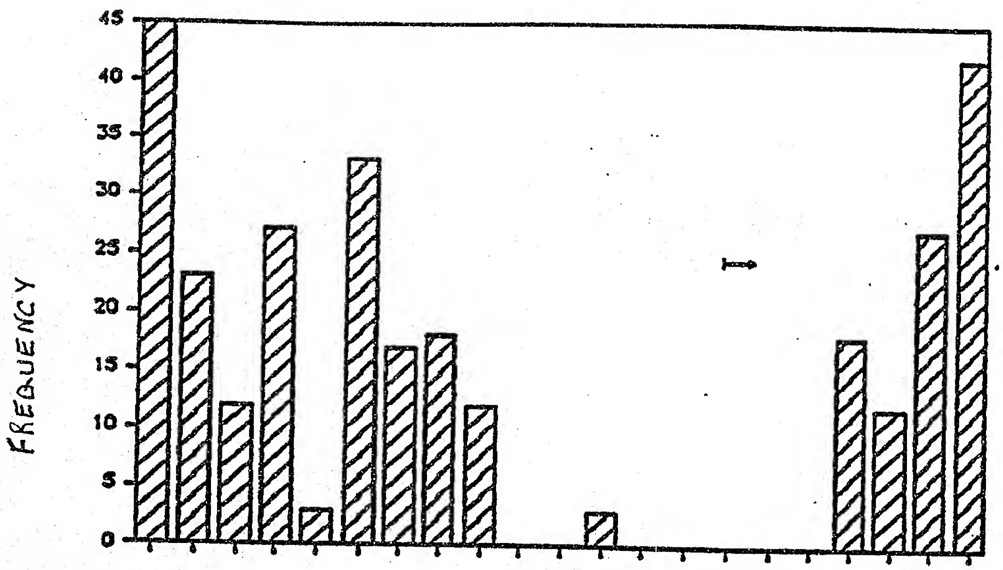

DIAGRAM 4: INHIBITION OF THE BONDING OF EcPhoA - Sc3D6 FUSION PROTEIN TO gp160 VIA HIV-1 POSITIVE SERA